Serologic evaluation of human microcystin exposure

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Introduction
Microcystins (MCYST) are among the most commonly detected toxins associated with cyanobacteria blooms worldwide. Biological evidence of human exposure is needed in order to evaluate potential MCYST-associated health effects. MCYST are detectable in free and bound forms in human serum. We will provide an overview of selected methods to detect biological evidence of exposure in humans, and will identify some uncertainties associated with interpretation of results.

Methods
We analyzed serum samples collected from MCYST-exposed patients after exposure events at Brazilian dialysis clinics during 1996 and 2001. We used a commercially available enzyme linked immunoassay (ELISA) method to detect free MCYST, liquid chromatography/mass spectrometry (LC/MS) to detect free MCYST, and gas chromatography/mass spectrometry (GC/MS) to detect 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB). MMPB is derived from both free and protein-bound MCYST by chemical oxidation, so it appears to represent total MCYST present in serum.

Results
Exposed patients provided blood samples for analysis after exposure. In a subset of 10 serum samples we found similar concentrations of free MCYST between the ELISA and LC/MS methods (Spearman $r=0.96$, $p<0.0001$). ELISA measurement of free MCYST was consistently lower than MMPB quantification of total MCYST. ELISA measured free MCYST as 8 – 51 % of total MCYST. Among the larger exposed population, we found evidence of free MCYST in patient serum for more than 50 days after the last date that documented MCYST exposure occurred.

Conclusion
MCYST are present in serum in free and protein-bound forms, though the nature of protein bound forms is uncertain. Analysis of serum samples for the presence of free MCYST may be performed in a cost-effective manner using screening assays such as the ELISA, but they underestimate total circulating concentrations. The relationship between free or total MCYST and absorbed dose is unknown due to limited knowledge of distribution and clearance. We found that free MCYST concentrations in patient serum may be detected for more than 50 days after the last documented exposure occurred. However, it is possible that patients experienced continued MCYST exposure by some route that was undetected during this study. Research is urgently needed to elucidate the human toxicokinetics of MCYST, in part to determine how measured serum levels can be used to estimate MCYST exposure.